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PRINCIPAL INVESTIGATOR: **John W. Olney, M.D.**

PI ADDRESS: **Dept. of Psychiatry, Washington University School of Medicine**
4940 Audubon Avenue, St. Louis, MO 63110-1081

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Systemic treatment of adult rats with a high dose of pilocarpine (pilo) or with a low dose of lithium (li) followed by a low dose of pilo causes a cholinotoxic syndrome consisting of persistent seizures and a disseminated pattern of seizure-related brain damage. Our preliminary observations suggested that this type of brain damage resembles that seen in rats following persistent seizures induced by other convulsants such as kainic acid, which, in turn, resembles the excitotoxic type of damage that the excitatory transmitter glutamate is known to cause. To further assess the similarities between the pilo or li-pilo cholinotoxic syndromes and other seizure-related brain damage syndromes, we studied the progression of electrophysiological (surface and depth recordings), metabolic (2-deoxyglucose autoradiography) and neuropathological (light and electron microscopic) changes in various regions of rat brain during the acute period in which seizure activity and brain damage occur. In addition, we measured enzyme markers for the gamma aminobutyric acid and cholinergic transmitter systems in various brain regions and studied [^{3}H]-glutamate receptor binding by autoradiography at chronic time points after rats had sustained seizure-related brain damage from li-pilo treatment. Some of these studies are ongoing and remain to be completed but the results obtained so far are consistent with the hypothesis that the neuropathological changes seen in the cholinotoxic syndrome could be mediated by an excitotoxic mechanism, i.e., by excessive activation of the glutamate transmitter system.

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Weldon T. Price   7-5-89
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TABLE OF CONTENTS

Introduction

Systemic injection of kainic acid (KA), an excitatory amino acid (EAA), induces distinctive behavioral effects in rats. Within 15 minutes of injection rats display stereotypic behaviors such as compulsive grimacing, staring and posturing. Within 30 minutes they progress from occasional wet dog shakes to repetitive rearing seizures which include copious salivation and sustained clonus of head and forelimbs, continuing for many hours. Rats that have seized in this manner for several hours suffer brain damage disseminated throughout the limbic system that is identical to the distinctive cytopathology induced by exposure of neurons to glutamate (Glu) or other EAA in vivo or in vitro (1,2,3,4).

In previous studies we have observed a pattern of behaviors, seizures and brain damage similar to that induced by KA, in rats following intra-amygdaloid injection of cholinergic agonists such as pilocarpine (pilo), oxotremorine and carbachol or acetylcholine (ACh) esterase inhibitors, physostigmine or neostigmine(5,6). Systemic injection of pilo, either alone or preceded by lithium (li), induces this same pattern of seizure-related brain damage (SRBD)(5). Activation of muscarinic cholinergic receptors has been implicated in the initiation of seizure activity in these studies in that pretreatment with atropine prevents both the seizures and related brain damage (7,8). However, since non-cholinergic convulsants also induce seizure-related cytopathological changes and these changes are indistinguishable from the excitotoxic type of neurodegenerative changes that Glu is known to cause, we propose that the actual toxic action against neurons in all SRBD syndromes may be mediated by Glu or a related endogenous excitotoxin that may be released by the seizure activity. A similar hypothesis might be entertained regarding the cholinotoxic syndrome associated with potent anticholinesterases such as soman. The studies of pilo-treated animals described herein are preliminary to studies which will be conducted on soman-treated animals.

Here we describe studies designed to clarify the nature and mechanisms of pilo-induced brain damage. A major aim of the present research was to use surface and depth electrode recordings, 2-deoxyglucose (2-DG) autoradiography and light and electron microscopy to study the pathologic progression of events occurring in the rat brain following subcutaneous (sc) administration of pilo. This was accomplished in an extensive series of experiments in which we compared the electrophysiologic, metabolic and neuropathologic responses associated with high dose pilo treatment with those associated with low-dose li-pilo treatment. In addition, we conducted an autoradiographic analysis of Glu receptor binding in li-pilo-treated rats, reasoning that if excessive glutamergic stimulation is responsible for the SRBD, there may be a substantial loss of Glu receptive neurons which would be reflected autoradiographically by a decrease in Glu receptors.

A third approach to the study of SRBD was to determine whether enzyme markers for certain transmitter systems are altered in the brains of li-pilo-treated rats. Brain concentrations of glutamic acid decarboxylase (GAD), the synthetic enzyme for gamma amino butyric acid (GABA), and choline acetyl transferase (CAT), the synthetic enzyme for ACh, were monitored over a 12-week period following li-pilo-induced SRBD. GAD was chosen for study as it has long been thought that seizure activity may differentially damage GABAergic inhibitory neurons. If this were the case, it would not be inconsistent with our hypothesis that Glu receptors mediate SRBD, since many GABAergic neurons are Glu-receptive cells. We chose to study CAT, even though we have no basis for predicting either a differential loss or preservation of neurons containing CAT, because we thought it would be worthwhile to follow the status of the cholinergic transmitter system in a comprehensive manner following induction of a cholinotoxic syndrome.

Materials and Methods

Adult Sprague-Dawley rats weighing about 300 g, bred and raised in the animal quarters of the Department of Psychiatry at Washington University Medical School on a 12-hour, light-dark cycle with free access to food and water, were used in all experiments. Li and pilo (Sigma Chemical, St.

Louis, MO) were administered subcutaneously (sc) in one of the following treatment regimens: 1.) 3 meq li followed after 24 hr by 30 mg/kg pilo, 2.) 400 mg/kg pilo without li pretreatment. In addition, 6 rats were injected sc with 12 mg/kg KA (Sigma Chemical, St. Louis, MO) in order to compare the electrographic and neuropathologic aspects of the KA syndrome with those produced by pilo. Control rats comprised 3 groups: 1.) saline only, 2.) 3 meq li followed in 24 hrs by saline, 3.) saline followed in 24 hrs by 30 mg/kg pilo. All animals that seized persistently for at least 2 hrs and were to survive at least 24 hrs were administered diazepam (10 mg/kg sc) 3 hrs and again 4 hrs after the onset of seizures to arrest the seizure activity, thereby assuring an acceptable survival rate. These animals were hand fed with liquid formula until they were able to function without aid.

Electroencephalography

For electrographic studies, animals were anesthetized with halothane and concentric bipolar electrodes (Rhodes Medical Instruments, Tujunga, CA), or bipolar stainless steel twist electrodes were stereotactically placed in various regions according to the coordinates of Pellegrino et al. (9). Depth sites monitored in various animals included the hippocampus, entorhinal cortex, amygdala, lateral septal nucleus, caudate-putamen, nucleus accumbens, ventral globus pallidus, substantia nigra and ventrobasal thalamus. Surface electroencephalographic (EEG) activity was recorded from Teflon-coated stainless steel wires implanted in the inner table of bone above the motor cortex. Additional stainless steel wires were positioned adjacent to the frontal sinus and served as ground and indifferent references. All electrodes were led to Amphenol pins in a connector board and the entire apparatus was affixed to the skull with jeweler's screws and dental acrylate. Each animal had 2-4 sites monitored. After surgery animals were housed in individual plexiglass cages and were allowed 1 week's recovery before experimental manipulation. Electrode placements were confirmed histologically at the termination of the experiment.

At the time of study, animals were connected to a Grass Model 7D polygraph and EEG signals were amplified and displayed using 7 P5 AC preamplifiers and driver amplifiers. Animals were observed for 20-30 min prior to drug injection and for up to 6 hrs after.

2-Deoxyglucose Autoradiography

For metabolic experiments, the quantitative [^{14}C]-2-DG autoradiographic technique of Sokoloff et al. (10) was used to measure local cerebral glucose utilization. Prior to experimentation, animals were fasted overnight. On the day of an experiment animals were anesthetized with halothane and polyethylene catheters were inserted into the femoral artery and vein. The animals were then loosely restrained for purposes of restricting movement. Body temperature was monitored via a rectal probe and kept at 36-37°C with a heat lamp. Animals were allowed to recover from anesthesia for 4 hrs before experimentation.

At the time of experiment, animals were injected with pilo sc. After the first episode of forelimb clonus, [^{14}C]-2-DG (60 mCi/kg) was administered by intravenous infusion over 30 sec. Timed 0.1-ml arterial blood samples were taken at 1/3, 3/4, 1-1/4, 2, 3, 5, 7-1/2, 12, 20, 30 and 40 min after 2-DG administration. The blood samples were centrifuged immediately and plasma radioactivity and glucose concentrations were measured. Animals were anesthetized with pentobarbitol 45 min after 2-DG administration and perfused with phosphate-buffered 1% paraformaldehyde fixative via an intracardiac cannula. The brains were rapidly removed, frozen and sectioned in a cryostat.

Brain sections (20 μm) were mounted with [^{14}C]-methyl-methacrylate standards (Amersham, Arlington Heights, IL) and exposed to Kodak X-AR film for 7 days. Brain radioactivity and local glucose utilization were determined in brain regions of interest by quantitative autoradiography using a computerized densitometer. Separate measurements of left- and right-sided structures were

made and the means of these measurements were pooled for subsequent analysis. Comparisons of experimental animals and controls in all experiments in this report were made using serial t-tests. Differences at a 95% or greater confidence level were considered significant.

Histopathology

Animals were killed by intracardiac perfusion-fixation under halothane anesthesia with phosphate buffered 1% paraformaldehyde and 1.5% glutaraldehyde 4 hrs after the initiation of seizure activity. The brains were removed and cut into 1-mm slabs which were additionally fixed by immersion in 1% osmium tetroxide for 24 hrs. These slabs were processed through graded concentrations of ethanol, cleared in toluene and embedded in Araldite. For light microscopy, 1-μm sections were cut on an MT-2 Sorvall Ultratome and stained with methylene blue-azure II. Regions of interest for ultrastructural analysis were sectioned from the same blocks. Silver-gold sections were mounted on a formvar film suspended over a 1 x 2 mm slot grid, stained with lead citrate and uranyl acetate and viewed in a JEOL 100B transmission electron microscope.

Receptor Autoradiography

Animals were sacrificed by decapitation after various survival times (1,2 and 3 wks). Their brains were quickly removed and frozen in powdered dry ice, then coronally cut into 10-μm sections on a Bright cryostat. In some instances brains were maintained at -80^{o} C before sectioning.

To study Glu receptor binding, sections were mounted on acid-washed, gel-coated coverslips and preincubated in 50 mM Tris acetate buffer, pH 7.25, at 4^{o} C for 60 min to remove endogenous Glu, air-dried and placed in xylene for 10 min. The sections were again air-dried, stored at -20^{o} C overnight (optional), then incubated for 10 min at 4^{o} C in Tris acetate buffer containing 25-1000 nM [^{3}H]-Glu (ICN, Irvine, CA). Nonspecific binding was studied by adding 0.5-1 mM unlabeled Glu to the incubation medium. Sections were rinsed for 3 sec in each of a graded series of ethanol-buffer solutions (30,50,70,95 and 100%), air-dried, exposed against ultrofilm (Amersham, Arlington Hgts.,IL) in light-tight cassettes and stored at 4^{o} C for 7 days. The films were then developed and the density of Glu binding in various brain regions measured with the Bioquant microdensitometric image analysis system (R&M Biometrics, Nashville,TN). Brain areas analysed at 5 coronal levels were cingulate, frontal, retrosplenial, piriform, parietal, temporal, occipital and insular cortices; caudate putamen; nucleus accumbens; nucleus of the lateral olfactory tract; CA-1, CA-2 and CA-3 regions of hippocampus; dentate gyrus; substantia nigra; dorsolateral and ventroposterior thalamus; medial and basal nuclei of amygdala.

Neurochemistry

For all neurochemical experiments, rats were allowed to survive 1-12 weeks following li-pilo-induced seizure activity. At time of sacrifice the animals were decapitated and their brains rapidly removed. Samples of neocortex, hippocampus, striatum, thalamus and piriform cortex-amygdala were dissected out, weighed and homogenized in ice-cold 50-mM Tris HCl buffer to which Triton X-100 (0.2%) was added. The homogenate (150 mg/ml) was centrifuged (10,000 g for 10 min at 4^{o} C). The resulting supernatant was collected and assayed in triplicate for CAT and GAD by the methods of Fonnum (11,12). Unless otherwise noted, all chemicals were obtained from Sigma Chemical, St. Louis, MO.

CAT

Fifty μg homogenate was incubated in medium containing 20mM ethylenediamine tetraacetic acid, 300 mM sodium chloride, 8mM choline chloride, 0.2% Triton X-100, 100mM physostigmine and

200mM [^{3}H]-acetyl coenzyme A (ICN Irvine, CA) in 50mM sodium phosphate buffer, pH 7.4, in a total volume of 82 µl for 30 min at 37° C. To stop the reaction, incubation tubes were placed inside 20-ml liquid scintillation counting vials and the contents washed into the larger tube with 5 ml 100mM sodium phosphate buffer, pH 7.4 at 25° C. Two ml of a saturated solution (1.5%) of sodium tetraphenyl borate in washed heptanone was then added, and the scintillation vials were tightly capped and vigorously shaken for 30-45 sec to extract the [^{3}H]-ACh salt into the organic phase. Ten ml of toluene-based fluorescent beta scintillation counting cocktail was added and radioactivity determined by a Packard TriCarb liquid scintillation analyzer. Control tubes contain either 1) no homogenate or 2) homogenate but no physostigmine and have 1 unit acetyl cholinesterase added.

GAD

Homogenate (150µg) was placed in 1µl medium containing 2 mM L-[1-^{14}C]-Glu, 0.05% bovine serum albumin, 0.2% Triton X-100, 1 mM mercaptoethanol and 0.4 mM pyridoxal phosphate in 50 mM sodium phosphate buffer pH 6.5. Each tube was filled with nitrogen gas and carefully sealed with 50 µl 2N sulfuric acid and then connected by a 6-cm length of tygon tubing to a second tube containing 50 µl hyamine hydroxide. Pairs of tubes were incubated for 60 min at 37° C. The acid was then flicked down into the homogenate mixture and the tubes remained at 25° C for 45 min to allow acid-generated gaseous [^{3}H]-carbon dioxide to be captured by the strong base. The hyamine hydroxide tubes were then placed in 20-ml liquid scintillation counting vials and their contents washed out with 2 ml 95% ethanol. Ten ml of a toluene-based fluorescent liquid scintillation counting cocktail was added and radioactivity determined by a Packard TriCarb liquid scintillation analyzer. Control tubes contained no homogenate or contained homogenate which had been boiled for 10 min.

Results and Discussion

Electrophysiologic, Metabolic and Neuropathologic Studies

Ten different surface and depth sites were monitored in 25 animals. No spiking or organized electrographic seizures were observed in any of the animals whether they were experimentals or controls. Figure 1 presents typical electrographic tracings from pilo-treated and KA-treated animals.

Using depth electrode recordings, we identified the nucleus accumbens in the ventral forebrain as the site of origin of seizure activity induced either by high dose pilo or li-pilo treatment. Seizure activity then spread rapidly to involve numerous limbic and related brain regions. Once initiated, electrographic seizures persisted for hours. Increased glucose utilization was found in most brain regions during the period of continuous seizure activity. The greatest increases were found in the ventral pallidum, globus pallidus, hippocampus, entorhinal cortex, amygdala, lateral septum, substantia nigra, ventrobasal and mediodorsal thalamus and frontal motor cortex (see Table 1). Animals sustaining seizures displayed a disseminated pattern of neural degeneration not involving globus pallidus or ventral pallidum but otherwise coinciding with the above pattern of enhanced glucose utilization. No consistent correlation was observed between the pattern of brain damage and known regions of high muscarinic cholinergic receptor density. Ultrastructurally, there was massive swelling of dendrites and somata and sparing of axons. These are the same cytopathologic changes associated with various other sustained seizure syndromes and they also resemble the excitotoxic type of damage Glu is known to cause(4).

The cholinergic seizure-brain damage syndrome closely resembled that induced by systemic KA, especially in behavioral aspects, but there were some differences in electrophysiologic and

metabolic manifestations. During KA seizures, electrographic changes were first detected in the hippocampus, whereas they were first detected in the ventral forebrain region in pilo seizures. Pilo also induced metabolic activation of ventral forebrain sites not activated by KA. The cytopathology associated with the two syndromes appeared identical in type but not in pattern, the cholinergic model being characterized by much greater neocortical and slightly less hippocampal damage.

By neuropathologic, electrophysiologic or metabolic criteria, the cholinotoxic syndrome induced by li-pilo (3 meq/kg plus 30 mg/kg) was indistinguishable from that induced by high dose pilo (400 mg/kg) alone. Moreover, the type of cytopathology observed is indistinguishable from that we have described in association with other seizure syndromes, which, in turn, is indistinguishable from the known excitotoxic effects of Glu(4). This is consistent with the interpretation that brain damage associated with pilo or li-pilo seizures may stem from a secondary activation of the glutamergic excitatory transmitter system. While a similar neurotoxic syndrome is produced by li-pilo and high dose pilo treatment, we are impressed that the former provides substantial advantages over the latter for studying mechanisms of cholinergic neurotoxicity. It consistently results in status epilepticus and a predictable pattern and degree of acute brain damage without a high incidence of acute mortality; this permits one to harvest groups of brain-damaged animals surviving weeks or months after seizure so that biochemical and receptor binding studies can be performed on their brains. After exhaustive efforts, in fact, we have concluded that it is not feasible to conduct such studies on high dose pilo animals because all such animals die within hours or a few days of treatment even if given large doses of diazepam after they begin seizing.

Autoradiographic receptor binding studies

The method we use for studying [^{3}H]-Glu receptor binding is similar to that of Monaghan and Cotman (13) except that we process the brain sections through xylene before incubation in [^{3}H]-Glu and we rinse the sections in a graded series of ethanols following [^{3}H]-Glu incubation. The xylene step eliminates a smudging artifact that otherwise confounds the autoradiograms and rinsing with ethanol increases the amount of specific binding that is retained without altering the ratio of specific to nonspecific binding. Since specific binding is increased by our method, we are able to generate strong autoradiographic images by exposure of brain sections to ultrofilm for only 7 days instead of the customary 21 days.

The autoradiographic data generated thus far are highly preliminary and pertain to only 3 animals at each survival time. Differences in binding between control and experimental animals were apparent by visual inspection of autoradiograms but we were unable to complete a quantitative analysis of the autoradiograms at this time due to malfunctions of the video camera in our Bioquant image analysis system. The camera has been returned to the manufacturer for repairs and quantitative data will be collected as soon as the camera is returned to us.

One, 2 and 3 weeks after li-pilo treatment there was a substantial reduction in [^{3}H]-Glu receptor binding throughout many brain regions. Reductions in [^{3}H]-Glu binding were most pronounced in regions that typically sustain damage following pilo treatment.

Neurochemical Studies

In the hippocampus, piriform cortex-amygdala and thalamus of li-pilo rats, GAD activity was significantly decreased in the 1-3-wk period following treatment but returned to and remained at control levels in the 4-12-week interval (Table 2). In the striatum and neocortex of li-pilo rats, GAD activity was not different from control levels throughout the 12 weeks following treatment. CAT activity was significantly decreased in striatum and piriform cortex-amygdala but was unchanged in hippocampus and thalamus throughout the 12-week period following li-pilo

treatment. In the neocortex, CAT activity was elevated in the 1-4-wk-interval and returned to control levels by 12 wks following li-pilo treatment.

These findings do not provide any immediate insights into the mechanism of neurotoxicity, although they are entirely consistent with the excitotoxic hypothesis, since the various neural elements destroyed by the excitotoxic process do not have anything in common except that they receive excitatory amino acid inputs. Thus, one might expect loss of either GAD- or CAT-containing neurons that lie postsynaptic to glutamergic terminals. If such neurons are intrinsic to a region in which excessive seizure-mediated release of Glu occurs, they may be destroyed. If such neurons project to other brain regions, there will be a loss of GAD or CAT both in the region of intrinsic neuronal loss and in the terminal fields. An increase in neocortical CAT concentrations for several weeks following li-pilo treatment is interesting and presumably reflects some kind of reactive change in the cholinergic system induced by li-pilo treatment and/or the associated seizures.

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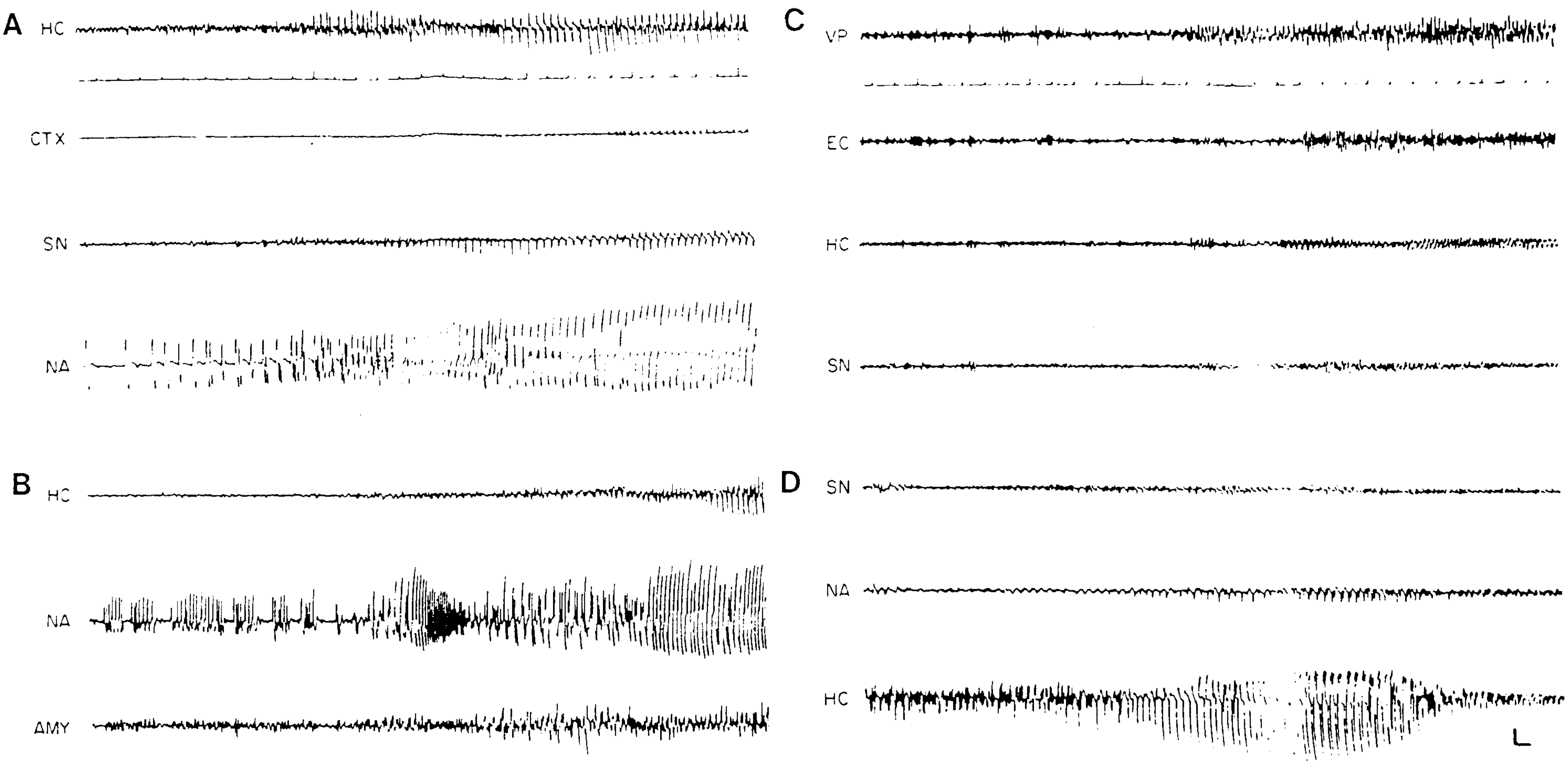

Fig. 1. Pattern of electrographic seizure onset in two animals (A and C) treated with lithium-pilocarpine and one animal (B) treated with high-dose pilocarpine. For comparison the pattern of electrographic seizure onset from an animal treated with kainic acid (12 mg/kg sc) is shown in (D). The traces display the first organized electrographic seizure in each animal. HC, hippocampus; CTX, motor cortex; SN, substantia nigra; NA, nucleus accumbens; AMY, amygdala; VP, ventral pallidum; EC, entorhinal cortex. Calibration bars: (A) HC = 1.5 mV, CTX = 0.5 mV, SN = 1.0 mV, NA = 0.5 mV; (B) 0.5 mV for all; (C) VP = 1.0 mV, EC = 0.5 mV, HC = 1.0 mV, SN = 0.25 mV; (D) SN = 1.0 mV, NA = 0.5 mV, HC = 1.5 mV. Time = 1 s for all.

Table 1. Glucose utilization (μmoles/100g/min) in pilocarpine-treated rats expressed as mean ± standard error of the mean (S.E.M.).

	Lithium-pilocarpine		Control		Pilocarpine	
	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.
Frontal cortex	295.67	25.50	73.77	5.41	323.97	39.57
Caudate	275.77	23.21	83.20	6.30	[illegible]	[illegible]
Medial accumbens	174.57	16.75	70.10	7.03	189.70	41.98
Lateral accumbens	175.90	16.23	68.47	5.88	187.80	26.71
Pyriform cortex	321.13	21.41	73.00	6.66	312.73	28.10
Corpus collosum	113.13	15.59	17.00	2.47	117.93	16.44
Lateral septum	253.50	20.47	51.03	5.29	275.50	36.67
Medial septum	156.00	14.70	68.60	5.98	173.53	29.61
Ventral diagonal band	138.97	18.49	66.40	3.43	163.57	31.13
Ventral pallidum	289.00	24.19	55.73	7.56	271.42	36.83
Olfactory tubercle	314.17	27.06	73.87	8.10	300.55	42.53
Globus pallidus	236.83	24.35	42.40	4.65	238.20	35.65
Horizontal diagonal band	144.00	27.25	65.10	4.10	156.30	32.67
Anterior cingulate cortex	240.63	30.30	89.60	2.77	295.17	55.13
Dorsal hippocampus CA1	172.83	13.41	35.03	8.24	249.70	37.43
Dorsal hippocampus perforant path	221.30	18.75	43.87	6.57	263.40	34.52
Dorsal hippocampus dor. dentate	213.27	18.49	47.83	8.71	278.43	37.98
Dorsal hippocampus ventral dentate	208.93	19.83	33.83	3.02	248.03	37.67
Dorsal hippocampus CA3	232.07	17.22	45.47	8.79	250.71	52.30
Amygdala	255.30	20.32	50.10	7.20	284.17	28.90
Ventrobasal thalamus	264.83	17.30	65.97	5.90	333.87	58.17
Medial dorsal thalamus	291.60	23.33	54.13	9.41	326.37	32.48
Sensory cortex	258.60	27.46	93.00	13.47	370.13	55.68
Hypothalamus	135.37	15.61	46.93	6.85	124.50	22.44
Hilum dentate	193.50	22.22	39.33	6.68	229.37	32.07
Habenula	144.00	11.23	62.13	9.56	190.87	27.04
Retrosplenial cortex	203.87	25.64	72.13	7.31	284.80	46.59
Visual cortex	215.93	18.36	68.10	5.80	285.67	44.09
Auditory cortex	235.20	16.71	116.65	5.36	308.33	47.26
Entorhinal cortex	260.90	25.87	43.90	5.96	275.27	22.99
Subiculum	256.97	22.04	46.13	6.88	290.80	29.35
Ventral hippocampus CA1	230.532	14.56	34.30	4.49	263.80	29.86
Ventral hippocampus CA3	242.10	22.47	43.37	6.88	252.37	31.41
Ventral hippocampus dentate	274.43	33.40	52.10	7.11	281.00	39.51
Substantia nigra	271.23	11.83	48.60	4.97	301.57	37.91
Medial geniculate	231.43	23.15	99.47	5.63	317.57	47.13
Dorsal lat. geniculate	69.27	7.72	51.13	6.04	100.57	19.25
Medial posterior thalamus	65.10	2.07	45.03	6.03	77.33	18.47
Superior colliculus	72.53	1.33	65.67	3.80	94.17	23.69
Periaquaductal gray	91.77	4.00	56.47	5.92	119.40	28.36

Table 2. Concentrations of GAD and CAT (pmoles/mg/min) in adult rats 1-12 wks after li-pilo treatment as compared to age-matched controls. Data are expressed as mean ± S.E.M. Asterisks indicate significant differences between control and li-pilo data as determined by Student's t tests (95% or greater confidence levels). (N = 6 per group.)

	1 wk		2 wk		3 wk		4 wk		12 wk	
	control	li-pilo	control	li-pilo	control	li-pilo	control	li-pilo	control	li-pilo
GAD										
Striatum	26.1±1.2	26.1±1.3	26.9±0.5	27.9±1.0	25.3±0.7	25.8±1.3	28.9±2.0	24.5±0.9	22.2±1.1	24.2±1.4
Neocortex	25.9±1.8	25.5±1.2	27.1±0.9	28.9±1.3	27.8±1.1	26.9±1.0	29.0±1.5	29.9±1.6	23.0±1.7	27.0±1.8
Hippocampus	26.4±0.9	22.0±1.3*	28.3±0.7	23.4±0.8*	29.3±1.0	24.5±0.8*	26.9±1.0	26.9±1.0	21.7±1.3	23.0±1.1
Piriform cortex and amygdala	29.1±1.3	23.1±4.3*	27.6±0.7	24.7±0.9*	29.2±1.1	22.9±1.3*	27.3±1.3	25.0±1.7	22.8±2.1	26.0±2.5
Thalamus	31.5±1.4	26.9±0.9*	34.2±1.1	28±0.9*	34.2±3.2	31.2±2.0	34.7±6.2	39±3.2	29.5±1.7	27.3±1.3
CAT										
Striatum	499±59	384±36*	364±16	278±35*	431±22	355±20*	432±22	306±43*	366±32	305±27*
Neocortex	140±8	206±18*	118±10	140±12	120±19	189±15*	119±22	142±34	103±7	92±8
Hippocampus	183±20	193±15	158±18	134±20	169±15	167±5	161±8	150±14	145±11	138±14
Piriform cortex and amygdala	291±24	220±19*	251±22	172±11*	282±16	223±13*	222±14	175±8*	203±14	173±14*
Thalamus	182±20	184±26	156±14	145±20	195±20	212±27	190±14	162±13	151±12	144±12